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Francisella tularensis periprosthetic joint infections diagnosed with growth in cultures

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Abstract

Tularemia caused by *Francisella tularensis* is a zoonotic infection of the Northern Hemisphere that mainly affects skin, lymph nodes, bloodstream and lungs. Other manifestations of tularemia are very rare, especially with musculoskeletal involvement. Presenting in 2016, we diagnosed two cases of periprosthetic knee joint infections (PJI) caused by *Francisella tularensis* in Europe (one in Switzerland, one in Czech Republic). We only found two other PJI cases in literature, another knee PJI diagnosed 1999 in Ontario, Canada, and one hip PJI in Illinois, USA 2017. Diagnosis was made in all cases by positive microbiological cultures after 3, 4, 7, and 12 days. All were successfully treated, two cases with exchange of the prosthesis, one with debridement and retention, and one with repeated aspiration of the synovial fluid only. Antibiotic treatment was given between 3 weeks and 12 months wither either ciprofloxacin/rifampin or with doxycycline alone or doxycycline in combination with gentamicin.

Zoonotic infections should be considered in periprosthetic infections in particular in culture negative PJIs with a positive histology or highly elevated leucocytes in synovial aspiration. Here we recommend to prolong cultivation time up to 14 days, perform specific PCR tests, and/or do epidemiologically appropriate serological tests for zoonotic infections including that for *F. tularensis*.

Introduction

Most commonly isolated microorganisms in periprosthetic joint infections (PJIs) are staphylococci, streptococci, enterococci, Gram-negative rods, and anaerobic bacteria (1). However, 5-35% of PJI remain culture negative (1) either because of antibiotics given prior to diagnostic aspiration, the inability to detect a recognized PJI pathogen using currently available diagnostic methods, or because of difficulty to cultivate or otherwise identify fastidious microorganisms such as anaerobes, mycobacteria, or fungi.

Little is known about zoonotic infections in joint replacements. We report two PJI cases caused by *Francisella tularensis* diagnosed in Europe. We searched for positive cultures and specific serology for *F. tularensis* in our microbiological database in general and reviewed literature of other orthopedic infections caused by *F. tularensis*. Focusing on PJIs, we summarized their clinical and microbiological characteristics in a mini-review.

Methods

Culture and identification methods

University of Zurich: To extract bacteria from periprosthetic tissue, samples were vortexed using 4mm glass beads (Sarstedt, Nürmbrecht, Germany). After homogenization, samples were incubated under aerobic and anaerobic conditions on agar plates and in thioglycolate broth (BD, Allschwil, Switzerland) for enrichment. For aerobic cultivation Columbia sheep blood agar without antibiotics (BioMérieux, Mary-l'Etoile, France), Colistin nalidixin acid (CNA) blood agar (BioMérieux), MacConkey agar (BioMérieux), and Crowe agar (chocolate agar supplemented with bacitracin and isovitalex (Difco GC medium, Becton Dickinson)) were used. Brucella agar (anaerobic sheep blood agar plates with haemin and vitamin K1, BioMérieux),

kanamycin-vancomycin agar (laked sheep blood brucella agar plates with kanamycin and vancomycin, BD), and phenylethylalcohol agar plates (BD) were used for anaerobic cultivation (Whitley anaerobic workstation MG1000, Don Whitley Scientific, West Yorkshire, England). Agar plates were incubated for 7 days at 37 °C. Thioglycolate broth medium was inspected daily for cloudiness, then subsequently plated onto chocolate (aerob) and Brucella (anaerob) agar plates for further identification. If thioglycolate broth cultures were negative after 10 days of cultivation, blind subcultures plated on chocolate and Brucella agar plates were performed and cultivated for another 2-3 days. Any suspicious bacterial colony, will be analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using a Bruker MALDI Biotyper in combination with research-use-only (RUO) versions of the MALDI Biotyper software package (version 3.0) and the reference database V.3.3.1.0 (4613 entries) or later database versions.

Ceske Budejovice Hospital: Cultivation methods and duration of cultivations were done in a similar way than in Zurich.

Retrospective case finding

We searched for published PJI cases caused by *F. tularensis* using PubMed, Scopus, and Medline for an epidemiological investigation (searched keywords: Francisella, tular*, joint, arthritis, prosth*, osteomyelitis, bone, replacement).

Retrospective microbiological review

We reviewed our hospital databases at the Institute for Clinical Microbiology at the University of Zurich and the Ceske Budejovice Hospital for positive *F. tularensis* serology or cultures with association of bone and joint infection.

Results

Two cases in Europe in 2016

Case 1. An 84-year-old Swiss woman presented in July 2015 with chronic knee pain with reported onset since December 2014 after a right knee joint arthroplasty in 2002. Intermittently, she observed an erythema above the knee without swelling. Serum C-reactive protein (CRP) was elevated with 64 mg/L. Synovial aspiration of the right knee joint revealed elevated leucocytes of 11,850 cells/µL with dominance of neutrophils (80%) without any growth of microorganisms. X-ray showed no loosening of the implant but small tibial osteolysis. A PJI was suspected and the prosthesis was removed as the first surgery of a two-stage exchange of the prosthesis. At time of explantation of the prosthesis, five out of six tissue biopsies showed growth of *F. tularensis* on a blind subculture on day 12 after a blind subculture of thioglycolate broth inoculated on day 10, while inoculation on agar plates remained negative. Histology of a tissue biopsy of the recessus medialis revealed focal acute inflammation (dominance of neutrophils) and extended wear of the prosthesis (Supplemental Figure 1a and 1 b). Serology with elevated IgM and IgG was in line with a *F. tularensis* infection. We changed the empiric intravenous antibiotic treatment with amoxicillin-clavulanate to oral doxycycline for a total duration of six weeks after implant removal. After two weeks off antibiotics, the new knee prosthesis was successfully implanted. In the last follow-up two years later, the patient was feeling well and having a good quality of life, free of infection and a follow-up tularemia IgM titer went down from 232 to 111 (U/ml). As a potential acquisition of *F. tularensis* we thought of airborne transmission of contaminated dust while cleaning the near located rabbit barn, or a tick bite. However, the patient could not remember any tick bite. She remembered having an episode of fever and sore throats a few

145 months before the onset of knee pain. None of the 10 laboratory staff involved in
146 handling the culture-positive agar plates showed a seroconversion after two months.
147
148 **Case 2**. An 84-year-old Czech male had a history of right knee total arthroplasty in
149 2006 and PJI in the same joint caused by *Escherichia coli* in 2008 that was treated
150 with open synovectomy, mobile components replacement and retention of the fixed
151 components, along with antibiotic therapy. After this, he was asymptomatic for 8
152 years.
153 In July 2016, he presented with fever, abdominal pain and elevated inflammatory
154 parameters (CRP 166mg/L), but no source of infection was found. He was treated
155 with oral amoxicillin-clavulanate and discharged as fever and abdominal symptoms
156 rapidly resolved. Ten days later, he presented to an orthopedic clinic complaining of
157 increasing pain in the right knee where a large effusion had developed. Blood tests
158 have shown leucocyte count of 4.80×10^9cells/mL and CRP 98.4mg/L. The right knee
159 effusion was aspirated yielding over 70 mL of cloudy fluid, which showed highly
160 elevated leukocytes (++++ by microscopy, flow cytometry was not possible because
161 of high viscosity of the fluid) and no organisms. After four days of the aspirate
162 incubation, small colonies of Gram-labile (indifferent state) to Gram-negative
163 coccobacilli were seen on Columbia 5% sheep blood agar (Bio-Rad Corp., Hercules,
164 CA, USA). Because of their morphological appearance, *F. tularensis* was suspected
165 and 16S rDNA sequencing was performed from the colonies, which confirmed the
166 identification. Serology for tularemia had shown a 1:80 titer (total antibody;
167 microagglutination test, Bioveta Inc, Ivanovice na Hané, Czech Republic).
168 The knee X-ray was not suggestive of loosening of the implant and a
169 synovectomy and mobile components replacement with retention of the joint
170 prosthesis was recommended. The patient declined any surgical intervention, and

171 therefore he was treated with doxycycline 100 mg orally twice a day for 21 days, of
172 which the first 10 days in combination with gentamicin 240 mg intravenously once
173 daily. Two months later he presented with recurring effusion of the knee, and the
174 second re-aspiration has shown fluid with minimum white cells, no bacterial growth of
175 prolonged culture and negative 16S rDNA PCR. He was given another course of
176 antibiotics, this time ciprofloxacin 500 mg orally twice a day for 20 days.
177 During the follow up, the persistent small, pain-free effusion in his right knee was re-
178 aspirated after four, 10, and 24 months from the initial presentation. All aspirates
179 were unremarkable regarding cell-count, culture negative and 16S rDNA PCR
180 negative. His blood inflammatory markers were unremarkable as well. He did not
181 complain of any systemic symptoms and the knee X-ray did not detect any signs of
182 loosening. After 24 months, he was discharged from the clinic with advice to be re-
183 referred in case of any problems.
184 Personal history identified garden work and outdoor walks in tularemia endemic area
185 as the only risk factors for zoonotic infections. Given the abdominal symptoms,
186 intestinal form of tularemia was suspected to be the port of entry into the bloodstream
187 and secondary spread to the knee.
188 Operating room and laboratory staff involved in handling of the first aspirate and
189 culture positive agar plates were offered prophylactic course of doxycycline (n=7).
190 None of them developed any clinical symptoms of tularemia.
191
192 Retrospective case findings

193 Two other PJI cases (2, 3) and one osteomyelitis case (4) but without a joint
194 prosthesis were found in literature. Clinical and microbiological characteristics of all
195 four PJIs are summarized in Table 1. Three out of four PJI cases due to *Francisella*
196 *tularensis* presented in knee arthroplasty. All were detected by positive

microbiological cultures and confirmed by positive serology, three with confirmation result by molecular-genetic methods, and one with additional acute inflammation in histopathology as an indirect sign. All were successfully treated, two cases with exchange of the prosthesis, one with debridement and retention, and one with repeated aspiration of the synovial fluid only. Antibiotic treatment was given between 3 weeks and 12 months using either ciprofloxacin with or without rifampin or doxycycline with or without gentamicin.

Microbiological database research

Zurich. In 12 PJI cases with available *F. tularensis* serology -classified as culture-negative PJIs, *F. tularensis* serology remained negative as found in a retrospective analysis of the microbiology database. Regarding positive growth in tissue biopsies or blood cultures, only 7 other positive *F. tularensis* cultures were documented in the same period in Zurich but without any association with an orthopedic infection.

Ceske Budejovice Hospital. No data about tularemia serology are available from prosthetic joint infections. Between 2003 and 2015, only one positive blood culture out of 64 tularemia cases (1.6%) was detected (unpublished data, under review).

Discussion

Orthopedic infections caused by *F. tularensis* are very rare. Next to our two PJI cases in Europe, we only found two other PJI cases, one described in Canada in 1999 (2, 3) and one in the United States in 2017 (2, 3). In other zoonotic PJIs such as *Listeria monocytogenes* (5, 6), *Coxiella burnetti* (7), *Pasteurella multocida* (8), or *Brucella* species (9), there is an increase in reported orthopedic cases recently. In contrast to the other zoonotic pathogens, we have not found any diagnosed native joint arthritisand only one case of osteomyelitis –caused by direct inoculation from a cat bite after penetrative open injury in conjunction with *Pasteurella* infection (4). Biofilm formation in *F. tularensis* - as it has been documented in vitro and in the aquatic environment (10)– might be an important virulence factor in prosthetic material related infection.

Three out of four PJI cases described here were localized in the knee joint. Two of them had a lesion on the lower limb, suggestive an infected tick bite (11) leading to an ulceroglandular form of tularemia. Such a skin lesion – often presenting as a non healing ulcer – may persist for several months. *F. tularensis* bacteria disseminate via the lymphatic system to the regional lymph nodes and other tissues (11). Alternatively, PJI could be caused by a hematogenous spread of *F. tularensis* in transient bacteremia or introduced in a previously damaged tissue via migrating granulocytes or macrophages using a Trojan horse mechanism. All four cases had some type of previous inflammation in the affected joint arthroplasty so that we question the possibility of migration of macrophages into the joint for reason other than tularemia. Those macrophages, attracted to a minor injury in the prosthetic joint, may have incidentally brought in *F. tularensis* in their vacuolae (12, 13) and the infection had flared up after apoptosis. Due to the fact that two out of four patients

were hunters, transmission route could also be through exposure to blood or body fluid with contaminated meat (11). In Germany, between 2002 and 2016, 10 outbreaks of tularemia were associated with contact to wild animals in the context of hunting (14).

In all four PJI cases, diagnosis was made by culture-based methods in part with longer incubation times than expected in other pathogens more commonly isolated in PJI (1). In the Swiss case, diagnosis would be missed if cultures were stopped at day 10. It can be hypothesized that other tularemia cases may have been missed due to short cultivation time, difficulty to grow, and categorized as „culture-negative“ PJI” cases (15). Some of them may have resolved spontaneously, while others might have been inadvertently rightly treated with ciprofloxacin, used in some centers as a part of empirical therapy for culture negative PJIs.

The low numbers of positive *F. tularensis* cultures or serology both in the microbiological laboratory of Zurich and Ceske Budejovice illustrates the rare event of *F. tularensis* in general. Large case series of *F. tularensis* infections reported 14% (149/1034 cases, Turkey tularemia cohort) (16) and 20.8% positive cultures (21/101 cases, French cohort 2006 - 2010) (17). None of these cohorts, though, reported native or prosthetic joint or bone infections. In general, majority of clinical tularemia cases are diagnosed by antibody test and only minority by culture. The low sensitivity of the culture-based methods might be due to the current practice in many microbiology laboratories where less than 7 incubation days are standard or only anaerobic cultures respectively thioglycolate enrichment broths are kept for ten or more days.

The fact that all four so far reported tularemia PJI cases have been diagnosed by positive culture may signify that this is only a tip of the iceberg and in reality, there are more cases undiagnosed. However, no zoonotic pathogens as potential PJI pathogens to be searched for are mentioned in major guidelines (18-21).

Antibiotic treatment in our four cases was given between 3 weeks and 12 months with either ciprofloxacin/rifampin or with doxycycline alone or in combination with gentamicin. Antibiotics susceptibility has not altered over years and doxycycline, aminoglycosides, and fluoroquinolones alone or in combination are mainstay of therapy (22). There is only limited data on *in vitro* antibiotic susceptibility of *F. tularensis*; however, no major changes in susceptibility rates have been reported. Duration of antibiotic therapy of more common manifestations of tularemia usually does not exceed 3 weeks, however, there have been case reports of prolonged clinical course of tularemia with the need of repeated antibiotic therapy (14). In all four reported cases of tularemia PJI, the antibiotic therapy was prolonged or repeated, and two of the cases appeared not to respond to initial course of antibiotics.

There is also a significant public health and occupational health issue in case that *F. tularensis* is cultured in a routine bench in microbiological laboratory from unsuspecting sample. Every institution having different approach, all contacts were offered prophylactic doxycycline without seroconversion testing (Czech case), while only observation for seroconversion was performed in the other (Swiss case, described in (23)).

In summary, *F. tularensis* is capable to cause prosthetic joint infections as shown in this article but it is a rarely detected pathogen in this setting. Culture positive PJIs begin to be more reported in recent years, probably due to increasing numbers of

implanted arthroplasties and aging population (24), along with awareness of prolonged incubation time (25, 26) in initially culture negative PJIs, and a more active lifestyle including outdoor activities of people with joint replacements. Since *F. tularensis* is a fastidious growing organism, we hypothesize that multiple cases may have been missed and recommend to consider tularemia together with other zoonotic pathogens in culture negative PJIs with a positive histology or significantly elevated leucocytes in the synovial fluid. For that, we suggest to prolong cultivation time up to 14 days including aerobic cultures, and perform specific PCRs along with additional serological tests for zoonotic infections including *F. tularensis* in culture-negative PJIs. While advancements in the molecular biology techniques, such as new generation sequencing (19, 27), may refine the proportion of PJI without known pathogen, clinicians should consider both travel-related and endemic zoonotic infections in cases of true culture and PCR negative PJI and request specific antibody tests as an additional diagnostic step after blood and joint/tissue sampling for pathogens appropriate for their given geography and patients travel history.

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Tables and Figures

Figure legends

Figure 1. Histopathology of the knee PJI case in Zurich with acute inflammation with dominance of neutrophils at time of implant removal. In A: Tunica synovialis with florid granulocytic inflammation. Triangles indicate small clusters of neutrophils (HE staining, 200x). In B: Foreign body reaction to prosthetic material with diffuse histiocytic infiltration and multinucleated giant cells, indicated by asterisks (HE staining, 200x)

405 **Tables**

406 **Table 1.** Clinical characteristics of 4 patients with *Francisella tularensis* periprosthetic joint infections (PJIs).

Case no., country	**1 (23)**, Switzerland	**2**, Czech Republic	**3 (2)**, Ontario, Canada	**4 (3)**, Illinois, USA
Age (years), gender	84, female	84, male	68, male	77, male
Time of presentation	2016	2016	1999	2017
Affected joint	Knee	Knee	Knee	Hip
Immunosuppression	No	No	Rheumatoid arthritis (methotrexate)	No
Previous infection of the affected joint	No	*E. coli* PJI infection 8 years prior, cured with DAIR	*Enterococcus faecalis* early infection, cured	No, but recent (7 days) revision THR due to pain and limited range of movement
Potential source of acquisition	Housing of rabbits in next-door house (infected dust?)	No apparent exposure, but abdominal symptoms prior to the joint effusion suggestive of intestinal tularemia	Hunter, tick bite 6 months before arthroplasty	Hunter, no tick bite
Time to diagnosis after previous surgical intervention/revision	12 years	8 years	6 months	25 years Bullous lesion on the

Case no., country	**1 (23)**, Switzerland	**2**, Czech Republic	**3 (2)**, Ontario, Canada	**4 (3)**, Illinois, USA
				shin 1 year before onset of symptoms
Clinical findings	Erythema, joint pain, no fever	Fever, abdominal pain, confusion, painful knee effusion	Discharge from the joint, no fever	Fever, joint pain
CRP (mg/L)	81	98	NA	16
ESR (mm/h)	69	NA	47	96
Microbiology				
Positive cultures	Yes (6 out of 7), thioglycolate broth Sonication negative	Yes, Columbia 5% sheep blood agar	Yes, chocolate agar	Yes, 2 joint aspirates, Vitek cultures
Type of sample	Tissue cultures	Joint aspiration	Joint aspiration	Joint aspiration
Days of cultivation until growth	12 days (blind subculture)	4 days	3 days	7 days
Molecular method	16S rDNA and specific PCR negative in tissue sample 16S rDNA sequencing of growing pathogen positive	16S rDNA PCR and sequencing of the colonies grown on agar	Culture Sequencing: *F. tularansis tularensis* Biovar type B	NA
Serology for *F. tularensis*	IgM 232.6 U/ml (N <	1:80 titer	1:320 titer	Results reported as

Case no., country	**1 (23)**, Switzerland	**2**, Czech Republic	**3 (2)**, Ontario, Canada	**4 (3)**, Illinois, USA
	10U/ml) IgG 126.4 U/ml (N < 10U/ml)	(microagglutination)	(microagglutination)	positive from the lab, without stating any titers
Finding in histopathology	Acute inflammation, wear of prosthesis	NA	NA	NA
Surgical treatment	2-stage revision joint replacement	Repeated aspiration only	2-stage revision joint replacement	DAIR
Antibiotic treatment	6 weeks Doxycycline 100 mg twice daily	3 and 3 weeks Doxycycline 20 days 100 mg bd + gentamicin 10 days 240 mg od, followed by 20 days of ciprofloxacin 500 mg bd	6 months Ciprofloxacin/ rifampicin (unknown dose) (initially no response to ciprofloxacin in monotherapy)	12 months Doxycycline 100mg twice daily
Cure (time to follow-up)	2 years	2 years	More than 6 months	1 year
Number of health-care workers who took post-exposure antibiotic prophylaxis	0	7	Unknown	Unknown

NA, not available; Rx, treatment, DAIR – debridement, antibiotics, implant retention, CRP – C reactive protein, ESR – erythrocyte sedimentation rate

Data not containted in the original case studies were obtained by email communication from the corresponding authors of the cited papers